IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of:	
Rieping, Mechthild	Art Unit: 1656
Appl. No.: 10/733,776	Examiner: D. Steadman
Filed: December 12, 2003	Atty. Dkt.: 7601/80921
For: **Process for the Production of L-Amino Acids Using Strains of the Enterobacteriaceae Family**	Conf. No: 9536

Brief on Appeal to the Board of Patent Appeals and Interferences Under 37 C.F.R. 41.37

Commissioner of Patents
U.S. Patent and Trademark Office
Customer Service Window, **MS Appeal Brief - Patents**
Randolph Building
401 Dulany Street
Alexandria, VA 22314

Sir:

A Notice of Appeal from the rejection of claims 11, 14, 15, 19, 20, 22, 23, 25 and 28-34, was filed for the above-captioned patent application on April 27, 2007. Appellant hereby files an Appeal Brief in triplicate. In addition, a check is enclosed herewith that includes the fee for filing a Brief in Support of an Appeal as set forth in 37 C.F.R. § 41.31.

I. Real Party in Interest - 37 C.F.R. § 41.37(c)(1)(i)

The inventor named on the above-captioned application executed an Assignment transferring all right, title and interest in the application to Degussa AG on January 7, 2004. This Assignment was recorded at the United States Patent and Trademark Office on Reel 014682, Frame 0167 on June 2, 2004. Thus, the real party in interest in this case is Degussa AG.

II. Related Appeals and Interferences - 37 C.F.R. § 41.37(c)(1)(ii)

Appellant is not aware of any other appeals or interferences that will directly affect, be directly affected by, or have a bearing on the Board's decision in the present appeal.

III. Status of the Claims - 37 C.F.R. § 41.37(c)(1)(iii)

The claims now pending in the application are 11, 14, 15, 19, 20, 22, 23 and 25-37. Of these, claims 35-37 have been allowed and claims 26 and 27 have been objected to. The claims that have been rejected and which are being appealed are claims 11, 14, 15, 19, 20, 22, 23, 25 and 28-34. The appealed claims may be found in the Appendix of this Brief.

IV. Status of the Amendments - 37 C.F.R. § 41.37(c)(1)(iv)

All of the amendments made by Appellant have been entered into the application. The only amendments made after the receipt of a final rejection were: a) replacement of the word "L-threonine" in line 12 of claims 11 and 35 with the word "L-amino acid," and b) the replacement of the word "recovered" in line 1 of claims 22 and 23 with the word "isolated." These amendments have been entered.

V. Summary of the Claimed Subject Matter - 37 C.F.R. § 41.37(c)(1)(v)

There are two independent claims that are being appealed, claims 11 and 28. Claim 11 is directed to a method of producing an L-amino acid by culturing bacteria of the genus Escherichia and then isolating the amino acid. The claim requires that the bacteria used in the method have a *yjgF* open reading frame (ORF) with the nucleotide sequence of SEQ ID NO:1 and that this ORF has been modified by one or more methods of mutagenesis selected from: a) deletion of all or part of the *yjgF* open reading frame; b) insertional mutagenesis due to homologous recombination in the *yjgF* open reading frame; or c) transitional or transversional mutagenesis with incorporation of a non-sense mutation in the *yjgF* open reading frame. The modification must result in an increased production of L-amino acid by the bacteria relative to the amount of amino acid produced prior to mutagenesis. Finally, the claim requires that the *yjgF* open reading frame encode the polypeptide of SEQ ID NO:2. Support for claim 11 may be found in the specification on: page 2, lines 16-21; page 3, line 20-page 4, line 9; page 4, lines 15 and 16; page 7, lines 23-28; page 11, line 20- page 12, line 5; and in Example 4, page 25, line 21- page 26, line 22.

Independent claim 28 is directed to a method of producing an L-amino acid by culturing bacteria of the genus Escherichia under suitable conditions and then either recovering the L-amino acid and determining the amount recovered or isolating the amino acid.[1] The claim requires that the expression of the *yjgF* open reading frame of in the bacteria be eliminated by deletion of the entire *yjgF* open reading frame and that this open reading frame encode the polypeptide of SEQ ID NO:2. Support for this claim may be found in the specification on: page 2, lines 15-21; page 3, line 29- page 4, line 9; page 6, lines 17-21; and page 7, lines 23-28.

Dependent claims recite additional limitations for claims 11 and 28 but these limitations are not the subject of additional arguments in the present appeal (*i.e.*, Appellant intends that all claims stand or fall together).

VI. Grounds of Rejection to be Reviewed on Appeal - 37CFR § 41.37(c)(1)(vi)

The Examiner has rejected claims 11, 14, 15, 19, 20, 22, 23, 25 and 28-34 based upon the allegation that they are obvious in light of the combination of Volz (*Prot. Sci. 8*:24-28 (1999)) and Enos-Berlage (*J. Bacteriol. 180*:6519-6528 (1998)). Volz is cited as teaching that the function of the *E. coli yjgF* gene may be determined by analysis of its crystal structure and Enos-Berlage is cited as teaching a method of phenotypically characterizing *yjgF* negative mutants by culturing bacteria and analyzing the products made. Although the Examiner seems to accept that the references do not specifically disclose that mutations in *yjgF* affect amino acid production or disclose the isolation of amino acids, he alleges that these claim elements are inherently present. Thus, according to the Examiner, the references render the claimed amino acid production method obvious even though they do not expressly suggest such a method.

VIII. Argument - 37CFR § 41.37(c)(1)(vii

Rejection of Claims 11, 14, 15, 19, 20, 22, 23, 25 and 28-34 Under 35 USC § 103

Unless otherwise indicated, the following arguments apply equally to independent claims 11 and 28. Arguments are not separately presented for the dependent claims and it is intended that these claims stand or fall with the independent claims.

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[1] As discussed during the prosecution of this case, the term "isolated" requires that fermentatively produced amino acids be at least partially purified whereas the term "recovered" does not.

Comments Concerning Content of the Cited References

Appellant agrees with the Examiner that the Volz reference teaches that one approach to determining the function of the *E. coli yjgF* gene product is by analysis of its crystal structure. However, there are two important points that should be stressed. First, Volz is concerned with analysis of the normal gene product, not mutated forms of the product. Second, the reference never suggests that a loss of *yjgF* gene function leads to increased bacterial amino acid production or otherwise suggests that bacteria with mutations in *yjgF* might be cultured and used to make amino acids.

Enos-Berlage is concerned with metabolic pathways by which Salmonella bacteria make thiamine. It was found that bacteria that have mutations resulting in a loss of both a primary and an alternative pathway can still make thiamine if they have a further mutation that results in a loss of a functional *yjgF* gene. It is Appellant's position that this reference has no suggestions that bacteria containing a mutated *yjgF* gene should be used for fermentatively producing amino acids and, as discussed further below, there are statements in the reference that actually appear to suggest that bacteria containing such mutations should *not* be used in the production of amino acids, *i.e.*, that teach away from the claimed invention.

Isolation or Recovery of Amino Acids is Not Obvious from the References Cited

One of the main claim requirements that distinguishes Appellant's methods from the cited prior art is the isolation or the recovery[2] of amino acids from cultures of *yjgF* mutants. The Examiner seems to recognize that there are no express teachings in the cited references suggesting this step. However, he argues that merely lysing cells, centrifuging out cellular debris and collecting the supernatant, would be sufficient to meet the claim requirements. Since, according to the Examiner, this is disclosed in the prior art, the isolation or recovery of amino acids is inherent in the disclosure and Appellant's claimed methods are therefore obvious.

In response, Appellant submits that there are both factual and legal problems with this argument.

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[2] Claim 11 refers solely to the isolation of amino acids whereas claim 28 allows for either isolation or recovery. Although, for convenience, the arguments presented simply refer to "recovery," it will be understood that claim 28 actually requires both recovery and also a determination of the amount of amino acid recovered. Thus, merely collecting fermentation broth would not meet the requirements of claim 28, the collected material would need to also be assayed for amino acid levels.

1. Factual Considerations

Appellant can see no place in either cited reference where cells having a *yjgF* mutation were cultured, lysed, the lysate centrifuged and the resulting supernatant collected. The Volz reference is concerned with determining the crystal structure of the normal, unmutated *yjgF* gene product. There would be no need to culture bacteria with mutated forms of the gene to accomplish this. In fact, the use of mutant bacteria would be entirely incompatible with the objective set forth in the paper. i.e., analyzing the crystal structure of mutated, nonfunctional forms of the *yjgF* gene product would clearly not be a reasonable way for determining what the normal, functional, gene product does.

The Enos-Berlage reference is concerned with a problem of intermediary metabolism, *i.e.*, determining what mutations allow bacteria to make thiamine when normal pathways of synthesis have been lost due to other mutations (see abstract and first three paragraphs of the reference). PCR amplification and sequence analysis allowed the authors to conclude that mutations in the *yjgF* gene could confer this capability (see page 6520 of the reference, second column under the heading "Localization of yjgF mutations by PCR amplification"). However, Appellant does not see any suggestion that the authors lysed mutant cells, removed cellular debris and then kept the supernatant. Collecting supernatant as such would certainly not be inherent in carrying out PCR amplifications or sequence analysis. Appellant believes that procedures of this type normally involve the extraction of nucleic acids from cells and the elimination, not isolation or recovery, of amino acids.

2. Legal Considerations

Although inherency arguments are appropriate in novelty rejections, they are inappropriate for obviousness rejections.[3] The Examiner has argued that, even though the Enos-Berlage authors were unaware that there was a connection between increased amino acid production and mutations in *yjgF*, they inherently made and isolated amino acids in Salmonella and this renders the fermentative production of amino acids in different bacteria obvious. However, this reasoning is seriously flawed. How can a reference make the use of *yjgF* mutants for the fermentative production of amino acids in bacteria obvious, when it does not teach any relationship between mutation and increased amino acid production? Why would one of skill in

[3] In the present case, there are clear reasons why a novelty rejection could not validly be made. For example, the Enos-Berlage reference uses Salmonella bacteria rather than bacteria of the genus *Escherichia* and the Volz reference fails to disclose bacteria with mutated form of yjgF.

the art isolate or recover (and assay) amino acids from cultures of Escherichia bacteria when there are no teachings of such an isolation or recovery in the reference in connection with Salmonella bacteria? Appellant submits that patent law simply does not recognize inherent disclosures as a basis for an obviousness rejection.

The Teachings of Enos-Berlage and Volz are Incompatible with One Another

The Volz reference describes a study in which the crystal structure of the *yjgF* product was studied in an attempt to determine the biological function of the protein. Studying a protein from a mutated, nonfunctional, gene (the genes taught in Enos-Berlage) would clearly be incompatible with this objective. Similarly, Enos-Berlage reports that mutated Salmonella bacteria are capable of maintaining thiamine production *provided that* their yjgF gene is mutated. Based upon its teachings, the substitution of the normal *E. coli* form of the gene would make no sense. Thus, there is no motivation to combine the teachings of Enos-Berlage with those of Volz.

Enos-Berlage Teaches Away From the Use of yjgF Mutants for Amino Acid Synthesis for the Salmonella Mutants Studied

The Enos-Berlage reference is concerned with strains of Salmonella bacteria that have undergone mutations to eliminate metabolic pathways that are normally present and that, as a result, must be grown under special conditions. There is no indication that the conditions described would be conducive to amino acid production. In addition, the reference has statements that suggest that a loss of *yjgF* function due to mutation should decrease, not increase, amino acid production. For example, on page 6526, first column, second full paragraph under the "Discussion" section, the reference states:

> We suggest that the yjgF mutation results in the partial block of at least one step in isoleucine biosynthesis (by an as yet undefined mechanism) subsequent to the reaction catalyzed by threonine deaminase . . .

Thus, *yjgF* mutations are suggested to block, not promote, bacterial amino acid production. Appellant submits that the reference therefore teaches away form the methods claimed.

Conclusion

For the reasons discussed above, Appellant believes that the Examiner is mistaken in his rejection of claims under 35 USC § 103. It is therefore respectfully requested that the Honorable Board reverse the Examiner and remand this application for issue.

Respectfully submitted,

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VIII. Claim Appendix - 37 C.F.R. § 41.37(c)(1)(viii)

11. A process for producing an L-amino acid comprising:

a) culturing an enterobacterium of the genus *Escherichia* in a medium for a time and under conditions suitable for producing said L-amino acid; and

b) isolating said L-amino acid;

wherein the *yjgF* open reading frame of said enterobacterium has the nucleotide sequence of SEQ ID NO:1 and has undergone a modification by one or more methods of mutagenesis selected from the group consisting of: deletion of all or part of the *yjgF* open reading frame; insertional mutagenesis due to homologous recombination in the *yjgF* open reading frame; and transitional or transversional mutagenesis with incorporation of a non-sense mutation in the *yjgF* open reading frame, wherein said modification results in an increased production of L-amino acid by said enterobacterium relative to the amount of amino acid produced in said enterobacterium prior to said mutagenesis; and

wherein said *yjgF* open reading frame encodes the polypeptide of SEQ ID NO:2.

14. The process of claim 11, wherein said L-amino-acid is selected from the group consisting of: L-asparagine; L-serine; L-glutamate; L-glycine; L-alanine; L-cysteine; L-valine; L-methionine; L-isoleucine; L-leucine; L-tyrosine; L-phenylalanine; L-histidine; L-lysine; L-tryptophan; and L-arginine.

15. The process of claim 11, wherein said L-amino acid is L-threonine.

19. The process of claim 11, wherein said enterobacterium is of the species *Escherichia coli.*

20. The process of claim 11, wherein the expression of the *yjgF* open reading frame has been eliminated by the deletion of part of the *yjgF* open reading frame.

22. The process of claim 11, wherein said L-amino acid is isolated from said enterobacterium.

23. The process of claim 11, wherein said L-amino acid is isolated from said medium.

25. The process of claim 11, wherein culturing is performed using a batch process.

28. A process for producing an L-amino acid, comprising:

a) culturing an enterobacterium of the genus *Escherichia* in a medium for a time and under conditions suitable for producing said L-amino acid; and

b) either recovering said L-amino acid and determining the amount of said L-amino acid recovered or isolating said L-amino acid;

wherein the expression of the *yjgF* open reading frame of said enterobacterium has been eliminated by deletion of all of the *yjgF* open reading frame; and

wherein said *yjgF* open reading frame encodes the polypeptide of SEQ ID NO:2.

29. The process of claim 28, wherein said *yjgF* open reading frame has the nucleotide sequence of SEQ ID NO:1.

30. The process of claim 28, wherein said L-amino acid is selected from the group consisting of: L-asparagine; L-serine; L-glutamate; L-glycine; L-alanine; L-cysteine; L-valine; L-methionine; L-isoleucine; L-leucine; L-tyrosine; L-phenylalanine; L-histidine; L-lysine; L-tryptophan; and L-arginine.

31. The process of claim 28, wherein said L-amino acid is L-threonine.

32. The process of claim 28, wherein:

a) said *yjgF* open reading frame has the sequence of SEQ ID NO:1;

b) said L-amino acid is L-threonine; and

c) said enterobacterium is of the species *E. coli*.

33. The process of claim 11, wherein said L-amino acid is L-homoserine.

34. The process of claim 28, wherein said L-amino acid is L-homoserine.

IX. Evidence Appendix - 37 C.F.R. § 41.37(c)(1)(ix)

There is no evidence under 37 CFR §§1.130; 1.131 or 1.132 or other evidence entered by the Examiner that Appellant is relying upon on Appeal.

X. Related Proceedings Appendix - 37 C.F.R. § 41.37(c)(1)(x)

Appellant is not aware of any related proceedings for this application.